CLAIMS

1 Process for the production of virus in cell cultures comprising the steps of:

(a) preparing a culture of cells which are permissive to the virus and acceptable as a substrate for vaccine production;
(b) suspending the cells in a suitable medium followed by cell seeding at low densities;
(c) incubating the cell culture at 30 to 40 °C for an appropriate period of time;
(d) removing the medium from the cell culture of step (c) and inoculating with seed virus;
(e) incubating the cell culture of step (d) at 25 to 40° C for an appropriate period of time;
(f) removing the medium followed by washing the cells once or more times and replacing the medium;
(g) incubating the cell culture of step (f) at 25 to 40° C for an appropriate period of time;
(h) total or partial harvesting of culture supernatant containing virus with or without addition of stabilizer.
(i) optionally, carrying out multiple harvests of virus containing medium, at any desired interval, by replacing the removed medium and re-incubating the culture for an appropriate period;
(j) optionally, removing the cell debris and whole cells from the harvested virus;
(l) optionally, virus inactivation and;
(m) storing the virus at –45°C or lower.

2. Process according to claim 1 wherein the cells are selected from the group consisting of chicken embryo cells and mammalian cells which are interferon-producing cells when submitted to viral infection, such as chicken embryo fibroblasts (CEF), chicken embryo cells (CE), human diploid fibroblasts (eg. MRC-5), monkey kidney cells, fetal Rhesus lung (FRhL) cells.

3. Process according to claim 1 ~~and 2~~ wherein the cells are primary or any further passaged.

4. Process according to claim 1, ~~2 and 3~~ wherein the cell seeding is carried out at densities lower than $2x10^5$ cells/cm^2.

5. Process according to claim 4 wherein the cell seeding is carried out at densities in the range of $1x10^4$ - $2x10^5$ cells/cm^2.

6. Process according to claim 5 wherein the cell seeding is carried out at densities in the range of $1x10^4$ - $1x10^5$ cells/cm^2.

7. Process according to claim 1 wherein the culture is incubated, at steps e, g and i from 12 to 144 hours.

8. Process according to claim 7 wherein the culture is incubated from 12 to 72 hours.

9. Process according to claim 1 wherein stabilizer is used at step h.

10. Process according to claim 9 wherein the stabilizer is a substance acceptable as component in parenteral products and selected from the group consisting of human serum albumen (HSA), peptides, amino acids or proteins and mixtures thereof.

11 Process according to claim 1 ~~to 10~~ wherein the virus is a wild, attenuated or recombinant virus.

12. Process according to claim 11 wherein the virus is a Flavivirus.

13. Process according to claim 12 wherein the Flavivirus is Yellow Fever virus.

14. Process according to claim 13 wherein the Flavivirus is an attenuated Yellow Fever virus.

15. Process according to claim 14 wherein the Yellow Fever virus is the YF17D virus strain and substrains thereof.

16. Process for the production of recombinant virus in cell cultures comprising the steps of:
(a) preparing a culture of cells which are permissive to the virus and acceptable as a substrate for vaccine production;
(b) cell seeding at low densities;
(c) transfecting cells with nucleic acids by treatment with synthetic lipids or electroporation;
(d) incubating the cell culture at 30 to 40°C for an appropriate period of time;
(e) removing the medium from the cell culture of step (d) and inoculating with seed virus;
(f) incubating the cell culture of step (e) at 25 to 40°C for an appropriate period of time;
(g) removing the medium followed by washing the cells once or more times and replacing the medium;
(h) incubating the cell culture of step (g) at 25 to 40°C for an appropriate period of time;
(i) total or partial harvesting of culture supernatant containing virus with or without addition of stabilizer,
(j) optionally, carring out multiple harvests of virus containing medium, at any desired interval, by replacing the removed medium and re-incubating the culture for an appropriate period of time;
(l) optionally, removing cell debris and whole cells from the harvested virus;
(m) optionally, virus inactivation; and
(n) storing the virus at -45°C or lower.

17. Process according to claim 16 wherein the cells are selected from the group consisting of chicken embryo cells and mammalian cells each interferon-producing cells when submitted to viral infection, such as chicken embryo fibroblasts (CEF), chicken embryo cells (CE), human diploid fibroblasts (e.g., MRC-5), monkey kidney cells, fetal Rhesus lung (FRhL).

18. Process according to claims ~~16 and 17~~ Claim 16 wherein the cells are primary or any further passaged.

19. Process according to ~~claims 16, 17 and 18~~ Claim 16 wherein the cell seeding is carried out at densities lower than $2x10^5$ cells/cm^2.

20. Process according to claim 19 wherein the cell seeding is carried out at densities in the range of $1x10^4$ - $2x10^5$ cells/cm^2.

21. Process according to claim 20 wherein the cell seeding is carried out at densities in the range of $1x10^4$ - $1x10^5$ cells/cm^2.

22. Process according to claim 16 wherein the culture is incubated, at steps f, h and j from 12 to 144 hours.

23. Process according to claim 22 wherein the culture is incubated from 12 to 72 hours.

24. Process according to claim 16 wherein stabilizer is used at step i.

25. Process according to claim 24 wherein the stabilizer is a substance acceptable as component in parenteral products and selected from the group consisting of human serum albumen (HSA), peptides, amino acids or proteins and mixtures thereof.

26. Process according to ~~claims 16 to 25~~ Claim 16 wherein the recombinant virus is a Flavivirus.

27. Process according to claim 26 wherein the recombinant Flavivirus is Yellow Fever virus.

28. Process according to ~~claims 16 to 25~~ Claim 16 wherein the recombinant virus is a chimeric virus having a genome comprising nucleic acid sequences encoding at least one structural protein from one flavivirus and nucleic acid sequences encoding the remainder of the genome of another Flavivirus to make it functional.

29. Process according to claims 28 wherein the recombinant virus is a chimeric virus having a genome comprising nucleic acid sequences encoding at least one structural protein from one flavivirus and nucleic acid sequences encoding the remainder of the genome of YF 17D virus to make it functional.

30. Process for the production of Flavivirus vaccine in cell cultures comprising the steps of:
(a) preparing a culture of cells which are permissive to the virus and acceptable as a substrate for vaccine production;
(b) suspending the cells in a suitable medium followed by cell seeding at low densities;
(c) incubating the cell culture at 30 to 40 °C for an appropriate period of time;
(d) removing the medium from the cell culture of step (c) and inoculating with seed virus;

(e) incubating the cell culture of step (d) at 25 to 40° C for an appropriate period of time;
(f) removing the medium followed by washing the cells once or more times and replacing the medium;
(g) incubating the cell culture of step (f) at 25 to 40° C for an appropriate period of time;
(h) total or partial harvesting of culture supernatant containing virus with or without addition of stabilizer.
(i) optionally, carrying out multiple harvests of virus containing medium, at any desired interval, by replacing the removed medium and re-incubating the culture for an appropriate period;
(j) optionally, removing the cell debris and whole cells from the harvested virus;
(l) optionally, virus inactivation and;
(m) optionally, lyophilizing the vaccine composition of steps h, i, j or l to obtain a freeze dry form of the vaccine composition.

31. Process according to claim 30 wherein the cells are selected from the group consisting of chicken embryo cells and mammalian cells which are interferon-producing cells when submitted to viral infection, such as chicken embryo fibroblasts (CEF), chicken embryo cells (CE), human diploid fibroblasts (eg. MRC-5), monkey kidney cells, fetal Rhesus lung (FRhL).

32. Process according to ~~claims 30 and 31~~ Claim 30 wherein the cells are primary or any further passaged.

33. Process according to ~~claims 30, 31 and 32~~ Claim 30 wherein the cell seeding is carried out at densities lower than $2x10^5$ cells/cm^2.

34. Process according to claim 33 wherein the cell seeding is carried out at densities in the range of $1x10^4$ - $1x10^5$ cells/cm^2.

35. Process according to claim 34 wherein the cultures of steps e, g and i are incubated from 16 to 72 hours.

36. Process according to claim 30 wherein stabilizer is used in step h.

37. Process according to claim 36 wherein the stabilizer is a substance acceptable as component in parenteral products and selected from the group consisting of human serum albumen (HSA), peptides, amino acids or proteins and mixtures thereof.

38. Process according to ~~claims 30 to 37~~ Claim 30 wherein the virus is a wild, attenuated or recombinant virus.

39. Process according to claim 38 wherein the Flavivirus is Yellow Fever virus.

40. Process according to ~~claims 38 and 39~~ Claim 38 wherein the Flavivirus is an attenuated Yellow Fever virus.

41. Process according to ~~claims 39 and 40~~ Claim 39 wherein the Yellow Fever virus is the YF17D virus strain and substrains thereof.

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